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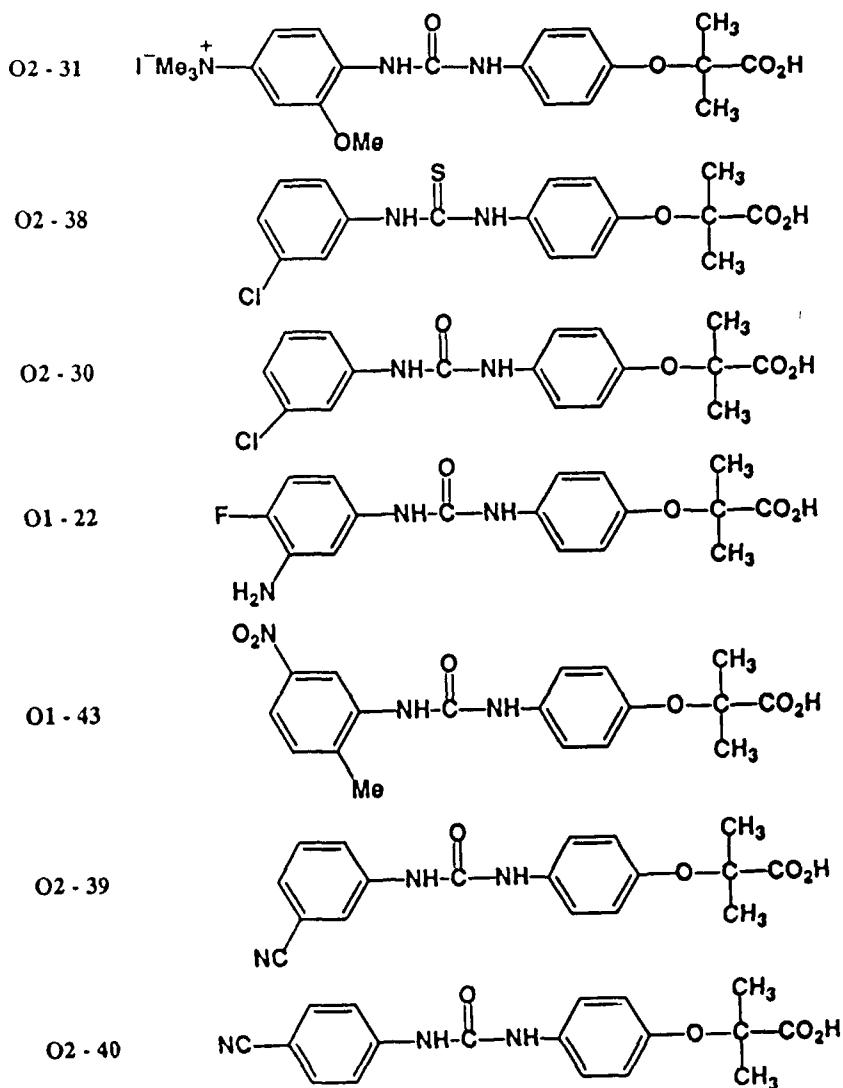
Department of the Navy  
 Office of the Chief of Naval Research  
 Grant No. N00014-90-J-1648  
 Principal Investigator: Thomas G. Burke, Ph.D.  
 March, 1992

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PERFORMANCE REPORT

A. Synthesis of Allosteric Effectors of Hemoglobin

Nine new LR16 analogues have been synthesized in sufficient amounts to allow for their characterization as modulators of the oxygen affinity of liposome-encapsulated hemoglobin. The structures of these new compounds are shown below and on the following page, while Table I on the next page summarizes the amounts of each of these compounds which we have in our possession.



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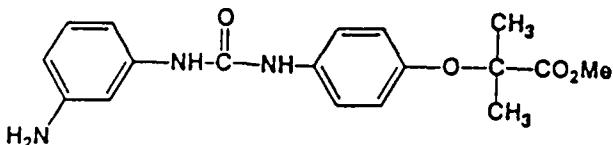
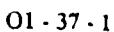
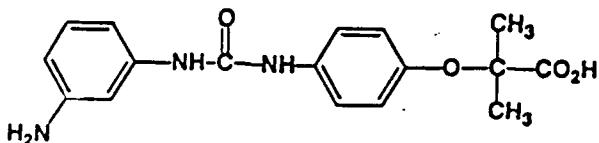
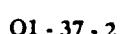


Table I: Molecular weights and available amounts of LR16 analogues.

<u>compound no.</u>	<u>molecular weight</u>	<u>available amounts</u>
02-31	402.38	52 mg
02-38	364.82	19 mg
02-30	348.76	29 mg
01-22	347.32	11 mg
01-43	373.32	43 mg
02-39	339.32	27 mg
02-40	339.32	42 mg
01-37-2	329.33	14 mg
01-31-1	343.34	20 mg

Among the compounds are nitro-substituted, sulfur-substituted, fluoro-substituted, and quaternized or permanently-charged analogues of LR16. These compounds have not been synthesized previously and thus it will be of interest to evaluate their ability to modulate the oxygen binding affinities of hemoglobin.

## B. Structural Verification

We have employed a fast atom bombardment mass spectrometer (FAB-MS) at the the Ohio State University Chemical Instrument Center to verify the structures of the synthesized compounds. Figure 1 found on the next page shows the FAB-MS spectra of the parent compound LR16 (Panel A) as well as that of the  $\text{BF}_3$  salt of 02-31 (Panel B). The observed molecular weights are consistent with the proposed stuctures. We are in the process of obtaining high performance liquid chromatographic (HPLC) equipment which will be used to evaluate sample purity.

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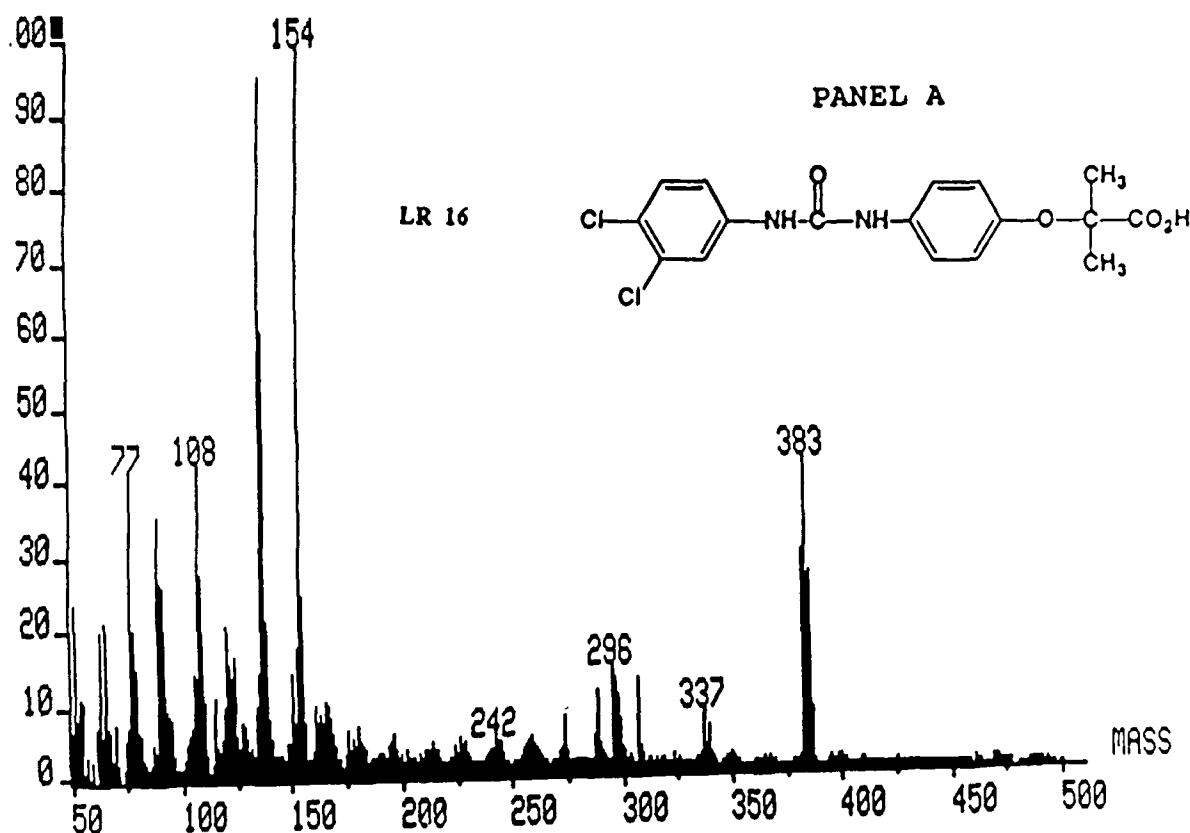
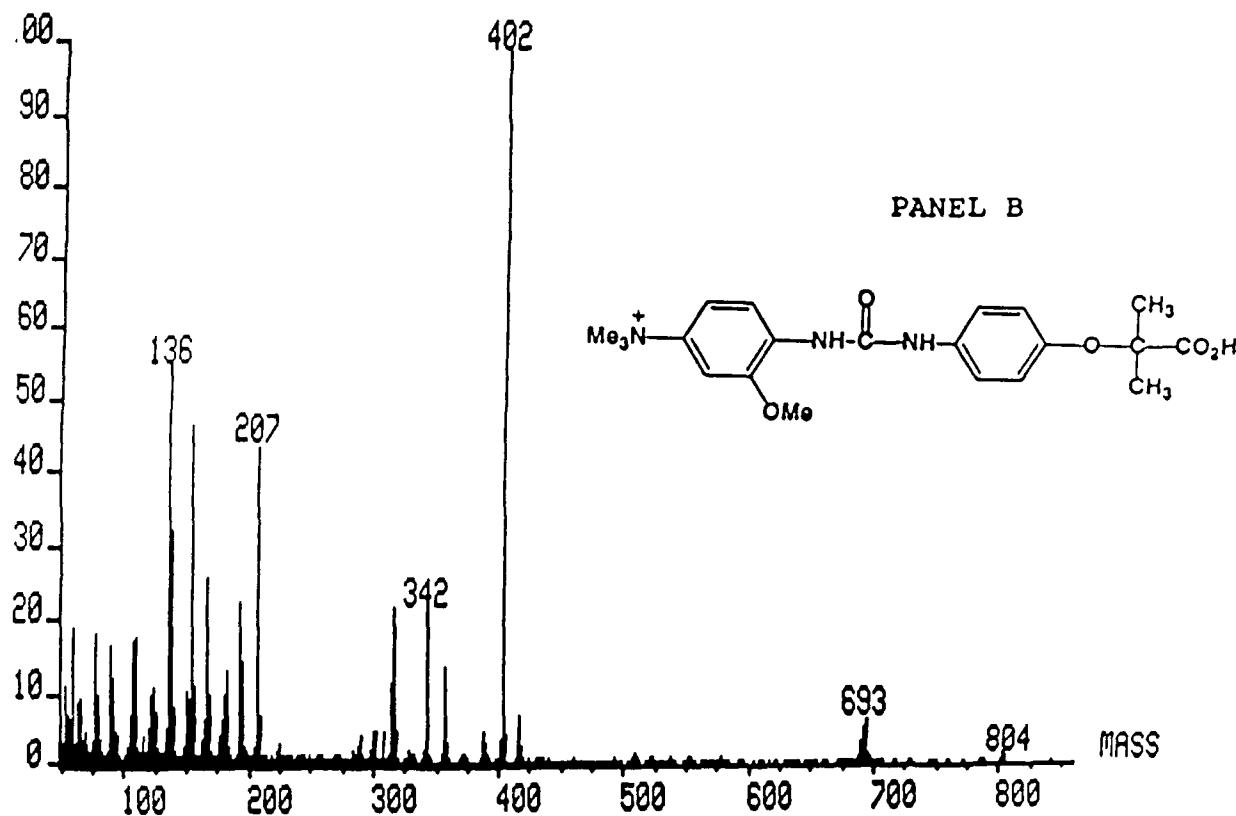


Figure 1. FAB-MS spectra of LR-16 analogues. Panel A is the spectrum of LR-16, while Panel B is that of its permanently-charged analogue.

C. Biological Evaluation of LR16 Analogues

In transitioning the research program to Ohio State University, it was necessary to buy a Hemox Hemoglobin Analyzer. This equipment arrived last month and has been set-up so that future testing of LEH materials will be completed here at OSU.

Dr. Alan Rudolph of the Naval Research Laboratory is in the process of sending us 20 ml. of a 26 gram % solution of purified human hemoglobin. In addition, Biopure has agreed to send to us a sample of purified bovine hemoglobin. When these samples arrive in mid-April, we will compare the abilities of the LR16 analogues to modulate the oxygen affinities of both human and bovine hemoglobins. Ms. Beena Kanekar, a third-year graduate student here at the OSU College of Pharmacy, will carry out these experiments as part of her doctoral thesis research.

We have also recently obtained approval from the Ohio State University Institutional Laboratory Animal Care and Use Committee to evaluate LEH/LR16 artificial blood formulations in small animals. The experiments will be performed here at the Ohio State University College of Pharmacy by Drs. Burke and Staubus. A copy of Animal Care and Use Committee Protocol approval is attached to this report.

THE OHIO STATE UNIVERSITY  
INSTITUTIONAL LABORATORY ANIMAL CARE AND USE COMMITTEE  
ACTION OF THE COMMITTEE

Original Review

Periodic Review

ANIMAL USE PROTOCOL:

92A0055      EVALUATION OF LEH/LR16 ARTIFICIAL BLOOD FORMULATION, T.G. Burke and  
A.E. Staubus, Pharmaceutics

The Institutional Laboratory Animal Care and Use Committee took the following action on the protocol cited above:

Approved

Disapproved

Approved with Modifications\*

\* Modifications requested by the Committee have been accepted by the Investigator(s) and, therefore, the protocol is APPROVED.

Approval of the protocol is for the period 04/01/92 through 03/31/95. The Committee will periodically contact investigators to seek information about approved protocols. Periodic contact for this protocol is next scheduled for 03/93.

The Investigator(s) shall immediately bring to the attention of the Institutional Laboratory Animal Care and Use Committee any changes proposed for the approved protocol as they relate to the care or use of laboratory animals. The Committee will decide whether the extent or type of changes proposed warrant formal Committee review. If such a review is deemed necessary, the chairperson shall schedule the review for the earliest feasible time.

Prior to the initiation of any projects you should contact the attending veterinarian or person in charge of animal care within the college in which your animals are to be housed. Additional information is generally required to comply with the specific college needs before the initiation of any projects. Failure to make such contact may delay the initiating of this project.

Certification of review and approval will be transmitted to external sponsors, as required, by the Research Foundation. **INVESTIGATORS ARE RESPONSIBLE FOR CONVEYING A COPY OF THIS DOCUMENT TO THEIR SPONSORED PROGRAM DEVELOPMENT OFFICERS.**

Date March 20, 1992

Signed  John O. Staubus  
(Chairperson)